Observations and Discussion

The present research work was designed to understand the effect of aqueous and ethanolic extract of *A. paniculata* and *E. viride* on thioacetamide induced liver cirrhosis and nephrosclerosis in albino rat, *Rattus norvegicus*. The results obtained in the present research work with respect to histological structure of liver and kidney in control rat, and histopathological changes produced in these two organs in rats intoxicated with thioacetamide for 4, 8 and 12 weeks and in thioacetamide intoxicated rats for 8 weeks after treated with aqueous and ethanolic extract of *A. paniculata* and *E. viride* are described in detail hereafter in this chapter.

A. Liver

**Histological and histopathological observations**

a. Histological observations – Control group

**Group 1:**

The liver of control rat was lobular. It was divided into number of lobes namely left, right and caudate lobe. Each of the lobes was further divided into sub lobes. Left lobe consisted of two lobes namely left lateral lobe and left median lobe. The right lobe was also consisted of right lateral lobe and right median lobe while caudate lobe was made up of papillary process, processus papillaris and caudate process.

H. E. stained sections of liver in control rat revealed normal histological architecture which are shown microphotograph (Plate No. 1 Figs.1 - 3). It was consisted of number of lobules called hepatic lobules separated from each other by septum of connective tissue. Each of the lobule was roughly hexagonal (diamond) in shape and consisted of cords of regularly arranged hepatocytes radiating from central vein. Hepatic cells were polygonal in shape with well defined boundaries and acidophilic cytoplasm. Each of the hepatocytes showed a round to oval, centrally placed blue coloured nucleus, with one or two nucleoli. On the either side of hepatic cords there were seen the blood sinusoids called hepatic sinusoids appeared as narrow spaces lined by flattened endothelial cells.
Observations and Discussion

and Kupffer cells. At the periphery of hepatic lobules portal triad was seen (Fig.3) consisting of hepatic artery, hepatic portal vein and bile duct. Narrow spaces called space of Disse or perisinusoidal spaces were observed in between the endothelial lining cells of sinusoids and the hepatic cord cells. Tiny channels called bile canaliculi were observed in between the hepatocytes. Hepatic stellate cells also called Ito cells were observed in the perisinusoidal spaces while Pit cells which were the lymphocytes were observed in periportal areas.

b. Histopathological Observations – Intoxicated group

Group 2.1. :

Light microscopic observation of the H. E. stained section of the liver of rat from above group showed significant degenerative changes in its structural components as shown in microphotograph (Plate No. 1 Figs. 4 - 7). Disorganization and disarray of hepatic cords was initiated. Hepatocytes were hypertrophied and irregular in shape. Dilation and disorganization of central vein and increased number of Kupffer cells was also noticed (Fig. 4). Nuclei were pyknotic in some of the hepatocytes while karyorrhexis and karyolysis in some of the hepatocytes was also observed. Increased number of binucleation was noticed in some of the hepatocytes. The foggy and cloudy hepatocytes were observed at certain regions (Fig. 5). Infiltration of mononuclear cells was the prominent change observed in the liver of rat from this group. Congestion and dilation of portal vein as well as sinusoidal dilation were initiated (Fig. 6). Necrosis of hepatocytes in some region was also evident (Fig.7).

Group 2.2. :

Highly significant histopathological alterations as compared to the previous group were observed in the liver of rat from this group which are shown microphotographically (Plate No. 1, Fig. 8 and Plate No. 2, Figs. 9 – 13). Severe disarray in hepatic cords was observed. Formation of pseudolobules with thick fibrotic septa and bridging centrolobular necrosis was appeared in (Fig. 8).
Increased fogginess and cloudiness of hepatocytes was also seen. Severe alterations in nuclear organization such as binucleation, pyknosis, anisokaryosis, and karyolysis were noted. Cytoplasmic vacuolation and granularity in cytoplasm was observed in some hepatic cells (Fig. 9). Significant dilation of portal vein and infiltration of blood cells in it was noted. There was severe damage to the central vein which was established by increased blood coagulation. Anisonucleosis was also seen in some hepatocytes (Figs. 10 and 11). Dilatation of sinusoidal spaces throughout the parenchyma, infiltration of mononuclear cells, nuclear hypertrophy, elongation of blood vessel were some of the other lesions seen in some regions (Fig. 12). Severe necrotic regions and focal necrosis with scar patches was also observed (Fig. 13). Hepatocytes were completely disturbed and their boundaries of demarcation were totally disappeared. Intensive parenchymal degeneration was also observed. Inflammatory cells were observed surrounding the portal triad. Considerable degenerative alterations with hydropic degeneration were seen in periportal region.

Group 2.3:

The alterations noticed in the structure of liver in TAA intoxicated rats for 12 weeks are shown in microphotographs (Plate No. 2, Figs. 14 – 16 and Plate No. 3, Figs. 17 – 20). The extent of the damage was more severe in the liver of rat from this group as compared to the rat from group 2.1 and group 2.2. The normal arrangement of hepatic cord was found completely disturbed (Fig. 20). Complete destruction of architecture of hepatocytes and focal necrotic patches (Fig. 18) with haemorrhage, dilation and obstruction of central vein with infiltrated blood cells was evident. Sinusoids were dilated and congested (Fig. 19). Many nuclei showed karyorrhexis and karyolysis (Fig. 14). Degeneration of hepatocytes and hepatic nuclei was more pronounced (Fig. 20). Elevated lymphocytic infiltration was apparent approximately in the bile duct. Blood cells were also found in sinusoidal spaces. Portal triad was disturbed and wall of portal vein was oedematous and dilated (Fig. 15). Bile duct hyperplasia (Fig. 16)
Observations and Discussion

and massive increase of inflammatory cells was observed around portal triad which shows inflammation (Figs. 15 and 16). Cystic bile duct filled with mucous and cellular debris and surrounded by inflammatory cell infiltrate and connective tissue was noticed surrounding the portal area. The periportal necrosis (Fig. 17), severe necrosis of hepatocytes and formation (Figs. 18 and 19) of hepatocellular adenoma (Fig. 20) was the most diagnostic hepatopathological change noticed in the rat from this group.

c. Histopathological Observations –treated group

Group 3.1.1:

As compared to the degenerative changes noticed in the various components in liver of rat from group 2.2, partial improvement in the degenerated structure was noticed in the liver of intoxicated rats after treated with aqueous extract of *A. paniculata* which are shown in microphotographs (Plate No. 3, Figs. 21 and 22). Restoration in liver architecture was substantiated by less nuclear hypertrophy, occurrence of less number of pyknotic nuclei, reduction in extension of karyorrhexis, karyolysis and normal arrangement of hepatic cells in cords. The improvement in the degenerated component was supported further as there was restoration in hepatic cell necrosis, disorganized central vein, disorganization in the portal area components etc. However sinusoidal dilation was still apparent, mild inflammation was still persisted but fibrotic septa were completely disappeared.

Group 3.1.2:

When the rats from group 2.2 were treated with ethanolic extract of *A. paniculata* the liver showed significant recovery in its structural components which are shown in microphotographs (Plate No. 3, Figs. 23 and 24). The improvement was more pronounced as compared to improvement noticed in the rat from group 3.1.1. The improvement was characterized by significant recovery in the arrangement of hepatic cords, central vein and hepatocytes structure which were as normal as in control rat. Though the nuclear alterations
such as pyknosis, karyorrhexis and karyolysis at few regions was still persisted most of the other regions showed normal structure. More restoration was found in centrolobular region than periarterial region. Restoration in the sinusoidal spaces was more pronounced as compared to the rat treated with aqueous extract of *A. paniculata*. Cytoplasmic vacuolation was not seen in this group of rat liver. Number of binucleated hepatic cells and Kupffer cells was found normal as in rat from group 1.

**Group 3.2.1:**

Regenerative changes observed in the various components in liver of TAA intoxicated rats for 8 week after the administration of aqueous extract of *E. viride* are shown microphotographically in Plate No. 4, Figs. 25 and 26. Arrangement of hepatic cords within the lobule was as normal as in normal rat. Similarly restoration in the organization of central vein and the portal area was also seen normal though the congestion of the central vein and hepatic portal vein was still persisted (Fig. 26). Most of the hepatic cells were normal. Though the necrosis of few hepatic cells was still evident, it was restricted to only few hepatic cells. Pyknosis and karyorrhexis were still apparent in few hepatic cells however its frequency was less. Sinusoidal network was quite normal when compared to the rat from group 2.2.

**Group 3.2.2:**

Restorative changes seen in liver of induced rat for 8 weeks after treated with ethanolic extract of *E. viride* are shown in Fig. 27 and 28. Arrangement of hepatic cords was normal (Plate No. 4, Fig. 27). Though the portal area appears normal, inflammation surrounding the portal area was still persistent (Fig. 28). There was partial recovery of the structure of central vein with only one exception that the congestion in the central vein was still apparent. Number of Kupffer cells was reduced when compared to the rat from group 2.2 and it was nearer to the rat from group 1. Majority of the hepatocytes assumed their normal
Observations and Discussion

shape (Fig. 27) however karyorrhexis was still noticed in some of the hepatocytes.

**B. Kidney**

**Histological and histopathological observations**

Normal histological structure of kidney in rat and the changes produced therein after intoxicated with thioacetamide at different periods of exposure and in intoxicated rats for eight weeks after treated with aqueous and ethanolic extract of *A. paniculata* and *E. viride* observed in H. E. sections under light microscope are shown in microphotographs Plate Nos. 5 - 9 and Figs. 29 - 66.

**a. Histological observations – Control group**

**Group 1 :**

The histological structure of kidney of rat is shown in microphotographs (plate 5 Figs. 29- 32). Each of the kidney was bean shaped, reddish brown coloured and was found covered with thin fibrous connective tissue capsule. Anatomically it was consisted of two regions the outer granular or dotted cortex and inner striated medulla. These two regions were arranged in the form pyramids called renal pyramids. The apices of these pyramids were directed towards concave side of the kidney at the region called hilus. The basic structural and functional unit of the kidney was the nephron which was consisted of number of distinct units found located both in cortex and medulla. Histologically the nephron was composed of renal corpuscle, the proximal convoluted tubule, loop of Henle, distal convoluted tubules and the collecting tubules and ducts. The renal corpuscles, the proximal convoluted tubule and distal convoluted tubules and few collecting tubules found located in the cortex while the ascending and descending limbs of loop of Henle and the collecting tubules and ducts were found lodged in the medulla region. Each renal corpuscle was rounded or irregular in shape and consisted of a tuft of blood capillaries, the glomerulus which was covered with double walled capsule, the Bowman’s
capsule. The outer parietal layer of the Bowman’s capsule was consisted of single layer of flat epithelial cells which were squamous. These cells enclosed a narrow space or the urinary space continued with the lumen of proximal convoluted tubule. The visceral layer was found surrounding the glomerulus. The visceral space was communicated with arteries of glomerulus. The proximal convoluted tubules were lined with single layer of tall cuboidal to columnar cells. They were with acidophilic cytoplasm. The nuclei were large, spherical or rounded and centrally located. The apices of these cells were provided with numerous microvilli forming brush border and the boundaries were not distinct. The lumen was small and uneven.

The distal convoluted tubules as compared to the proximal convoluted tubule were fewer in number in the cortex. They were lined with low cuboidal cells with faint cytoplasm and without microvilli at their apical side. The nuclei were round situated centrally or apically. The lumen was somewhat wider and elongated as compared to the proximal tubule.

Medulla region of kidney was consisted of closely packed thick and thin tubules of the loop of Henley and the collecting tubules. The thick and thin tubules were lined with flat to squamous epithelial cells and were with wider lumen. The thick tubules mostly appeared like the proximal or distal convoluted tubule and were with narrow lumen and clear cell boundaries when compared with proximal and distal convoluted tubules. The thin tubules showed structural resemblance to that of capillaries. The collecting tubules were lined with cuboidal epithelial cells with rounded nuclei and pale cytoplasm, cell boundaries were distinct.

b. Histopathological Observations – Intoxicated group

Histopathological changes observed in the kidney of rat intoxicated with TAA at different period (4, 8 and 12 weeks) of exposure are shown in microphotographs(Plate Nos. 5 - 9, Figs. 33 - 56).
Observations and Discussion

**Group 2.1.**: 

Histopathological changes seen under light microscope in the H. E. stained sections of kidney of rat from this group are shown in microphotographs (Plate No. 5, Figs. 33 – 36 and Plate No. 6, Fig. 37). Significant degenerative changes in the various components were observed. These degenerative changes were characterized by enlargement of glomerulus (Figs. 33, 34) with infiltration of blood cells (Fig. 33) increased glomerular cellularity (Fig. 35) tightly fitting the Bowman’s capsule with no periglomerular space in few glomeruli while (Fig. 33) increased urinary space in some of in Bowman’s capsules, elongation of tubules, karyorrhexis, pycnosis and karyolysis in tubular cells (Fig. 33). Hypertrophied tubular cells at certain regions, degeneration of tubular cells and their necrosis in some tubules, complete atrophy of some tubules (Figs. 34, 35), interstitial as well as tubular vacuolation, and accumulation of blood cells in the lumen of some tubules were also observed. Tubular epithelial cells in the medulla were hypertrophied, the tubular lumen was reduced in size as well as atrophy of the tubular cells in some region was noticed in the medulla (Plate No. 5, Fig. 36 and Plate No. 6, Fig. 37).

**Group 2.2.**: 

Highly significant histopathological alterations were observed in the kidney of rat exposed to thioacetamide for eight weeks which are shown in (Plate No. 6, Figs. 38 - 44 and PlateNo. 7, Figs. 45 - 47). Disorganization and rupture of glomerular architecture with infiltration of blood cells (Fig. 39), increase in periglomerular space (Fig. 38), elongation of tubules, disruption of tubular cells (Figs. 38, 39) with accumulation of blood cells in the lumen of ruptured tubules and vascular congestion were the most frequent alterations noticed in kidney of rat from this group. Some of the glomeruli were disrupted (Figs. 38, 40). Progressive karyorrhexis, pyknosis and karyolysis (Fig. 40) was also evident. Eosinophillic cast or hyaline cast formation in the lumen of necrosed tubules, sclerosed glomerulus (Fig. 41), accumulation of blood cells in haemorrhagic regions (Figs. 42, 43). Hyperplastic intimal sclerosis or onion peel
Observations and Discussion

proliferation of arteriole were the most significantly histopathological changes observed at this period of exposure. Increased necrosis with fibrosis at some tubular region, elongation of medullary tubules (Fig. 44), necrosis of tubular cells and complete destruction of tubules with fibrosis (Fig. 43) were also evident.

Group 2.3. :

TAA induced histopathological alterations observed in the kidney structure of rat from this group are shown in microphotographs (Plate No. 7, Figs. 48 - 52 and Plate No. 8, Figs. 53 - 56). Highly degenerative changes viz. reduction of glomerular size with increased periglomerular space (Fig. 48), focal segmental glomerulosclerosis, glomerular conjugation (Fig. 49), dilation, rupture and formation of haemorrhagic area, onion peel formation in the arterioles (Fig. 52), severe necrosis of tubular cells and complete destruction of cortical and medullary tubules, formation of hyaline cast in the lumen of tubules, and in glomerular region etc. Glomerular conjugation, interstitial fibrosis and formation of scar tissue were the most distinct histopathological alterations noticed in the kidney of rats when they were intoxicated with TAA for twelve weeks.

c. Histopathological Observations –treated group

Group 3.1.1 :

Light microscopic observation of the kidney of intoxicated rat for eight weeks after treated with aqueous extract of A. paniculata showed significant improvement in the intensity of histopathological alteration in some of the structural components of kidney which are shown in microphotographs (Plate No. 8, Figs. 57 - 59). The necrosis of tubuloepithelial cells was still persisted but the extent of area of necrotic region was reduced significantly (Fig. 57). Extent of tubular damage was also reduced. Some of the glomeruli and their periglomerular spaces appeared normal similar in control rat. The fogginess of glomeruli was decreased. Medullary tubules and their lining cells were quite normal (Fig. 59).
Group 3.1.2:

Highly significant improvement in the histological architecture noticed in the kidney of rat treated with ethanolic extract of *A. paniculata* when compared with the histological structure of kidney of TAA intoxicated rat (group 2.2) are presented photomicrographically in Plate No. 8, Fig. 60 and Plate No. 9, Fig. 61. As compared to rat from group 2.2 the dilation of the glomerulus was reduced and it was appeared almost normal in size. Periglomerular space was of normal size as in control rat. Glomerular blood capillaries were with normal organization and less number of blood cells was noticed. Reduction in tubular cell necrosis and restoration of these cells were also noted and tubular cells in most of PCT and DCT appeared normal though necrosis of these cells were noted at some regions. Tubular atrophy was still persisted in some regions but the extent of atrophic region was greatly reduced. Hyaline cast, scar tissue and interstitial fibrosis were not noticed. Medullary tubules and their lining cells were almost normal and the tubules were with normal lumen. Overall extensions of restoration of histological architecture in the kidney of rat from this group was much pronounced when compared with the kidney of rat from group 3.1.1.

Group 3.2.1:

Histological observation of kidney of rat from this group showed several regenerative changes in comparison with the histological architecture of kidney of rat from group 2.2 and the kidney structure appeared mostly normal as in kidney of normal rat (group 1). All these regenerative changes are shown in microphotographs (Plate No. 9, Figs. 62, 63). Restoration in the size, shape and the structure of the glomeruli was noticed and it was as normal as in control group. Stripping of tubular epithelial cells was still persisted but degree of degenerative changes was decreased. Tubular cells in most of PCT and DCT were normal however restoration of these cells was more pronounced in PCT than DCT and most of PCT were almost normal in structure when compared with the kidney of rat from group 2.2. Pyknotic nuclei were noticed in most of
Observations and Discussion

the tubular cells however at certain regions normal nuclei were observed. Formation of hyaline cast, scar tissue and interstitial fibrosis was not noticed.

**Group 3.2.2:**

Kidney of rat from this group showed several regenerative changes in its structure in comparison with the structure of kidney of rat from group 2.2 which are represented microphotographically in Plate No. 9, Figs. 64 – 66. Most pronounced regeneration was noticed in size and shape of glomeruli where they were as normal as in control. Almost all blood capillaries within the glomerulus were normal. Periglomerular space was almost normal. Tubular cell necrosis was still persisted but regeneration in these cells was largely initiated. Most of the PCT and DCT were dilated and hypertrophied. Congestion of blood vessels, interstitial fibrosis etc. was not observed. However, pyknosis and karyorrhexis were still persisted especially in medullary tubular cells.
Histopathological Discussion

Histopathology is the microscopic observation of diseased tissue and is used as an important tool to diagnose diseased tissue. Therefore, it is used extensively in medical sciences especially in anatomical pathology to detect the diseased part of a system in order to confirm the mode of treatment (Black, 2012). It is one of the important and sensitive tools with the help of which an accurate diagnosis of diseased portion of an organ is possible. Though, biochemical investigation can give an idea about the pathological condition of an animal, a clear and perfect picture of cytoarchitectural changes produced due to intoxication of a particular chemical substance is possible only by histopathological observations. Thus, the histopathological observation throws light on nature of structural changes and the extent of damage in an organ. As per Jayantha Rao (1982), histopathological study provides an insight into the tissue lesions to prove the manifestation of the deleterious effects of toxic substances.

Liver:

Liver is prime organ in the body. Besides its role in the endogenous metabolism of carbohydrates, proteins and lipids it also helps in the disposal of waste materials as well as in excretion of endogenous toxic substances and other xenobiotics. Thus, liver plays an important role in protecting and detoxifying the body from any harmful internal or foreign substances (Sallem et al., 2010).

As per Tolman (1998), there are more than thousands of xenobiotic substances which are potentially hepatotoxin. According to him the ability of any chemical compounds to produce histopathological changes in the structure of liver often depends upon the interaction of series of complex cellular processes that involved in its uptake, its biotransformation and cimination. Every drug therefore, is associated with the hepatic damage almost due to the role of liver in drug metabolism. Hepatic metabolism thus is a process which converts drug into intermetabolites that can be more easily excreted.
Observations and Discussion

Thioacetamide is one of such xenobiotic compounds, a potent hepatotoxic agent used in the present investigation to induce liver cirrhosis in albino rat. When rats were intoxicated with TAA for four, eight and twelve weeks, the liver showed varied degree of histopathological changes in its various components depending upon the period of intoxication. TAA induced histopathological alterations in the liver architecture have also been reported earlier by Alshawsh et al. (2011) and Kadir (2011) in male Sprague Dawley rats. They reported histopathological alterations such as loss of architecture, inflammation and congestion with cytoplasmic vacuolation, inflammatory cell infiltrations containing lymphocytes and mononuclear cells and centrilobular necrosis. Extensive damage characterized by severe necrosis, fatty degeneration, sinusoidal dilation and the presence of collagen bundles surrounding the lobules leading to thick fibrotic septa that disturbs the cellular architecture have been reported by Fazal (2014) in TAA induced male Wistar albino rats. Similarly, centrilobular necrosis along with various gradation of fatty changes comprising of small to large sized vacuoles, disarrangement of hepatic cells with blood accumulation in sinusoidal spaces have been reported by Sureshkumar (2015) in TAA induced Wistar albino rats of either sex. Similar histopathological changes have also been reported previously by Das (2012) in Wistar albino rats of either sex and by Wong et al. (2012) in Sprague Dawley rats. Intoxication of albino rats with TAA in the present investigation resulted into disorganization of hepatic cords and central vein, pycnosis, karyorrhexis and karyolysis of hepatic cells, cytoplasmic vacuolation, dilation and congestion of portal vein, sinusoidal dilatation and infiltration of mononuclear cells, focal necrosis with scar patches resulting into formation of hepatocellular adenoma. Thus, the histopathological results obtained in the liver of TAA intoxicated rats in the present investigation are more or less identical and are in accordance with the results obtained earlier by previous investigators in TAA intoxicated experiment animals.

Survey of existing literature pointed out that the studies on hepatotoxicity and related histopathological changes in rat/mice have been carried out by number of earlier researchers by using number of hepatotoxic substances.
Almost in all such studies more or less similar pattern of histopathological alteration have been obtained by these workers. Sahu and Ghosal (2007) reported hepatocellular steatosis or fatty changes, disarrangement of hepatic cord cells with pycnotic hepatocytes in the liver of mice intoxicated acutely with higher dose of piroxicam (non-steroidal anti-inflammatory drug) on the other hand sinusoidal dilations, pycnosis in many hepatocytes, severe hepatic degeneration and parenchymal cell necrosis in liver of mice treated repeatedly for 15 days with the same drug. Devendran and Balasubramaniam (2011) observed mild parenchymatous degeneration with granular appearance of hepatocytic cytoplasm, severe hydrophilic and vacuolar degeneration and significant cytoplasmic vacuolization with disseminated necrotic cells in liver of aflatoxin induced rat. Alterations in the hepatocytes mainly in the form of anisokaryosis, nuclear vesiculation, binucleation, cytoplasmic inclusions and swelling, hydropic degeneration and necrosis have been reported by Jarrar and Taib (2012) in lead induced males of the Wistar albino rats. Al-Mosaibin (2013) reported vacuolar cytoplasmic degeneration with pycnotic nuclei and polymorphism accompanied with blood sinusoids, congestion and oedematous of liver parenchyma, congestion of portal veins and accumulation of red blood cells with stasis and massive cellular exudates of lymphocytes and macrophage around the portal area as well as bile duct proliferation in liver of monosodium glutamate (MSG) induced rat. On the other hand he reported degeneration of centrolobular hepatocytes and their necrosis, disruption of blood sinusoids, decreased number of Kupffer cells and interstitial hemorrhage, pycnosis, karyorrhexis and karyolysis in some of hepatic cells, elongation of bile ducts as well as congestion of blood vessels in portal area in mice treated with acrylamide (ACR).

Toxicopathological effects of subacute dose of methoreate have been assessed by Patel et al. (2014) in liver of Wistar rats of either sex at three different dose levels. They reported dose dependent pathological alterations characterized mainly by degeneration, necrosis accompanied with hemorrhage and vascular congestion. Haouas et al. (2014) revealed diffused disorganization
Observations and Discussion

of hepatic cords; vascular congestion; dilation of sinusoidal capillaries, central vein and portal space; mild lymphoid and mononuclear infiltration within portal areas and central vein; fragmented chromatin material and vacuolar cytoplasm in liver of lead acetate intoxicated rat.

Kumbhare et al. (2015) made light microscopic study of liver in adult albino rat intoxicated with monosodium glutamate (MSG) and reported dose dependent histopathological alterations. Mild disturbances in liver architecture like enlarged and congested central vein with disturbed endothelial lining, marked variations in the nuclei in most of hepatic cells and lymphocytes infiltration in the portal area with low dose for 45 days while increased daily dose showed more injury like erosion of endothelial lining of central vein and other sinusoids, vacuolar and hydropic degeneration, pycnotic nuclei, marked loss of uniformity and regularity of the hepatic cords, hemorrhage with aggregation of R. B. Cs, indistinct cell membrane, fragmentation of the condensed chromatin material and break down of the nuclear membrane.

Luz et al. (2015) found marked cytoplasmic vacuolation, hydropic degeneration, hyperchromatic nuclei, hepatic necrosis evidenced by presence of hepatocytes with pycnotic nuclei and eosinophilic cytoplasm, aggregation of mononuclear cells in the hepatic parenchyma in the liver of male Swiss albino mice after intoxicated with diphenyl ditelluride. Dose dependent histopathological changes viz. mild to moderate sinusoidal dilation characterized by widening of hepatic capillaries, infiltration of inflammatory cells, necrosis of hepatic cells etc. have also been reported by Al-Forkan et al. (2016) in Wistar albino rats after subchronic exposure to arsenic. Combined effect of Cd and Hg has been evaluated by Dardouri et al. (2016) in male Wistar rats. They also reported severe liver damage including sinusoidal dilation of central vein, degenerated hepatocytes, focal necrosis, congestion of sinusoidal spaces, vacuolation, inflammatory cells infiltration, proliferation of Kupffer cells etc. Alkushi et al. (2018) injected C6H6Cl2 every other day to adult male albino rat and reported central vein dilation, hydropic degeneration characterized by marked
loss of uniformity and regularity in hepatic lobules, infiltration of inflammatory cells with congestion and dilation of portal vein, sinusoidal obliteration and pycnosis of nuclei in some of hepatic cells after 20 days. After 30 days treatment they found cellular degeneration, noticeable cytoplasmic granularity, hydropic degeneration, fragmentation of chromatin material, karyolysis, increase in the number of Kuffer cells, congestion and dilation of central vein, congestion and blockage of portal tract with increased number of mononuclear inflammatory cells infiltration. On the other hand massive degenerative changes manifested by marked hydropic degeneration, cytoplasmic granularity and vacuolation, severe karyolysis, marked increase in the pycnotic nuclei, mononuclear cell infiltration in the portal tract and single cell necrosis after 30 days of treatment.

Pycnosis, karyorrhexis and karyolysis of cell nuclei have been reported in most of the studies as prominent histopathological changes due to intoxication of certain hepatotoxin. According to AL – Mosaibin (2013) and Ortiz et al. (2006), these changes may indicate the loss of functional efficiency of the cells due to intoxication. Hypertrophy of the hepatocytes noticed in experimental animal after intoxication of any xenobiotic is due to the swelling of intracellular organelles especially mitochondria and endoplasmic reticulum as mentioned earlier by Majno and Jaris (1995) and Thompson (1995). Vacuolar and/or hydropic degeneration of hepatocytes are also the most frequently noticed histopathological changes. Abdel Hammed (2004) described these changes as a kind of cellular defense mechanism against injurious elements and preventing them from interfering with the biological activities of these cells. Cytoplasmic vacuolation containing fat droplets with pycnotic nuclei represents the response of the hepatic cells to the toxic substance (Kumbhare et al., 2015). According to Mollendrof (1973) hepatic toxicity of the toxic substances display itself in the form of cell vacuolation in its cytoplasm which is a cellular defense mechanism against injurious substances. These substances are segregated in Vacuoles and thus prevented interfering with cellular metabolism. As mentioned by Zhang and Wang (1984) cytoplasmic vacuolation is mainly a consequence of disturbances in lipid inclusions and fat metabolism. Lymphocytic infiltration towards the area
Observations and Discussion

of inflammation have been reported by most of the workers which might be the prominent response of the body tissue facing any injurious impact of toxicant as mentioned by Kumbhare et al. (2015). According to Joher et al. (2004); Liu et al. (2011); Sharma et al. (2010) and Haouas et al. (2014) lymphocytic infiltration and sinusoidal blood congestion are the indicators of liver damage due to toxic substances. Cheville (2009) reported that centrolobular hepatocytes are typically the primary sites of toxic injury as they have more surface receptors for toxic substance and less for oxygen, that might be the cause of severe damage noticed in this region in most of the earlier studies and in the present investigation.

The extracts of the different parts of the various medicinal plants have historically been used to treat many diseases. Many plants and polyherbal formulations claimed to have hepatoprotective activity. The hepatoprotective role of these plants is due to the antioxidant compounds like phenols, flavonoids, tannins etc. present in the parts of these plants are able to delay or inhibit the oxidative stress (Lata et al., 2014). The medicinal use of many plants as hepatoprotector has been reported in literature by many earlier investigators in the experimental animals. Girish et al. (2009) have tested the hepatoprotective effect of six polyherbal formulations (Liv 52, Livergen, Livokin, Octogen, Stimulive and Tefrolive) against paracetamol induced liver toxicity. They have reported that the pretreatment of low doses of Liv 52 and Livergen reversed the paracetamol induced liver toxicity while at higher doses, all six herbal formulations showed marked beneficial pharmacological, biochemical and histological effects. Hepatoprotective effects of ethanolic extract of Orthosiphon stamineus (medicinal plant commonly used in Malaysia for treatment of hepatitis and jaundice) in comparison with standard hepatoprotective drug Silymarin have been evaluated by Alshawsh et al. (2011) in TAA induced liver cirrhosis in rats. According to them histopathological examination showed good recovery in hepatocytes necrosis by extract as compared to Silymarin. Low dose showed regeneration of hepatocytes especially surrounding the fibrous tissue septa, significant increase in bile ductules, fat storing cells and Kupffer cells. Animals
treated with higher dose, showed nearly normal pattern of histological architecture of liver with an increase normal hepatocytes parenchyma, reduced fibrous septa and lymphocytes infiltration.

Hepatoprotective effect of Liv-first capsule (polyherbal Ayurvedic medicine) has been screened by Lima et al. (2010). They found potent hepatoprotective activity against CCl₄ induced hepatic damage in albino rat. According to them the hepatoprotective activity might be due to the antioxidant and free radical scavenging properties of the formulation. Effects of ethanolic extract of *Tinospora crispa* stem have been studied in TAA induced liver cirrhosis in rats by Kadir et al. (2011). They stated that *T. crispa* has a high antioxidant (apigenin) and radical scavenging activity that potentiated the TAA induced hepatotoxicity. Bayram et al. (2011) reported that in acute hepatic injury induced by CCl₄ in rat, dihydromycenol extracted from *Vitis vinifera* exhibits a potent hepatoprotective effect while geranyl formate, another extract from the same plant presents partial hepatoprotective effect at low doses and no hepatoprotective effects at higher doses. Acharya et al. (2012) observed necrotic lesions as well as extensive vacuolation in the liver of Wistar rats exposed to CCl₄ when compared with normal. On the other hand liver of rat treated with ethyl acetate extract of root of *Asparagus racemosus* along with CCl₄ was almost similar to normal in histology, size and staining properties as no vacuolation was observed and nuclei and nucleoli were clearly visible as in normal cells indicating the hepatoprotective effect of *A. racemosus*. According to them the liver of animal treated with crude methanolic extract, cytoplasmic vacuolation was significantly reduced while in animals treated with aqueous extract the protection was insignificant. They further added that the hepatoprotective activity of the roots of *A. racemosus* was due to the constituents present in the ethyl acetate fraction which was rich in flavonoids.

The hepatoprotective effect of aqueous extract of bark of *Dalbergia sisso* was studied by Narware et al. (2012). They found absence of necrosis and vacuoles which were noticed in hepatocytes of CCl₄ and paracetamol intoxicated
Observations and Discussion

rats. Histological observations made by Das and Kathiriya (2012) in liver of Wistar albino rats treated with aqueous extract of *Stevia rebaudiana* revealed disappearance of centrizonal necrosis, and no any centrizonal necrosis, greater reduction in periportal and centrizonal necrosis, greater reduction in periportal and centrizonal inflammation and no any centrizonal necrosis that were observed in the liver of TAA intoxicated animals that indicated the potential hepatoprotective activity of the aqueous extract of *S. rebaudiana*. Mohammad *et al.* (2013) reported that the degenerative changes caused in liver especially in liver cells in which these cells were seen shrunken with condensed nuclei, inflammation and infiltration of leucocytes, dilation of blood sinusoids of Cadmium treated rat liver were restored after given aqueous extract of *Zingiber officinale* (ginger) indicating its ameliorative effect on hepatic damage. Usha Rani *et al.* (2013) confirmed the hepatoprotective effect of methanolic extract of bark of *Nilgirianthus ciliatus* by the histopathological examination of the liver of treated rats against paracetamol intoxicated rats. The paracetamol treated group showed hemorrhage and necrosis in the liver parenchyma, vacuolated cytoplasm and collection of inflammatory cells while extract administered group showed almost normal architecture of liver as in normal and silymarin treated group of rats. They observed normal hepatocytes in control animals; intense centrilobular necrosis, vacuolation and fatty changes in paracetamol treated group and normal hepatocytes in extract and silymarin treated group.

Padmanabhan and Jungle (2014) have investigated the hepatoprotective role of HP – 4 (alcoholic extract of leaves of *Aloe vera*, *Bacopa Monniera*, *Moringa oleifera* and rhizome of *Zingiber officinale*) against CCl₄ induced hepatotoxicity in mice by histopathological examination of liver. Histological profile of the liver in CCl₄ treated animals showed severe intense centrilobular necrosis, vacuolization and macrovesicular fatty changes while the silymarin and HP-4 treated groups of animals exhibited significant liver protection against CCl₄ induced liver damage evidenced by the presence of normal hepatic cords, absence of necrosis and fatty infiltration. Suresh kumar *et al.* (2015) made comparative histopathological examination of liver sections in rats intoxicated
with TAA in rats treated with ethanolic extract of stem of *Shorea tumbuggaia* and silymarin followed by TAA intoxication and in rats and reported that in TAA intoxicated rat, liver showed hepatic cells with centrilobular necrosis along with fatty changes comprising of tiny to large sized vacuoles, disarrangement of hepatic cells and accumulation of blood in sinusoids. The liver sections from *S. tumbuggaia* treated animals showed lesser degree of visible changes similar to that observed in silymarin treated rat thereby suggesting the protective effect of the extract. Nitin *et al.* (2016) have reported that the histopathological changes induced by rifampcin can be significantly prevented by prior treatment of aqueous extract of *Vigna mungo* in rat. Alphonse (2017) reported that the hepatic steatosis fatty infiltration, hydropic degeneration and necrosis observed in CCl$_4$ treated albino rats were completely absent in the liver sections of the rats treated with ethanolic extract of stem and leaves of *Bacolepis nervosa* which suggested that the extract posses significant potential as hepatoprotective agent.

In the present investigation aqueous and ethanolic extract of *A. paniculata* and *E. viride* have been tested to know the hepatoprotective effect against TAA intoxicated albino rats. Rats intoxicated with TAA for eight weeks showed several histopathological changes in liver architecture. Severe disarray in hepatic cords, alterations in nuclear organization, cytoplasmic vacuolation, significant dilation of portal vein and infiltration of blood cells, severe damage to the central vein, dilation of sinusoids, severe necrosis of hepatic cells, formation of pseudolobules with formation of thick fibrotic septa and bridging centrolobular necrosis. TAA intoxicated rats after treated with aqueous extract of *A. paniculata* and *E. viride* showed improvement in the degenerated structure of liver in intoxicated rat. The improvement in the liver architecture was substantiated by lesser nuclear hypertrophy, less number of pycnotic nulei, reduction in extension of karyorrhexis and karyolysis normal arrangement of hepatic cords, restoration in hepatic cell necrosis; disorganized central vein and in portal area components. Histological observations of the sections of liver of TAA intoxicated rat after treated with ethanolic extract of both the plants reversed to large extent hepatic lesions produced by TAA and showed almost
normal architecture of liver comparable with the control animal. The improvement in the degenerated structure was more pronounced in intoxicated rat with ethanolic extract of both the plants when compared to the improvement noticed in intoxicated rat treated with aqueous extracts which was characterized by significant recovery in the arrangement of hepatic cords, central vein and hepatocytes structure was quite normal as in control rat. More improvement was noticed in centrolobular region than the peripoortal region. Number of Kupffer cells was nearer to the control rat. Restoration in the sinusoidal spaces was more pronounced as compared to rat treated with aqueous extract. No cytoplasmic vacuoles were seen and necrosis of the hepatocytes, formation of pseudolobules and thick fibrotic septa and bridging centrolobular necrosis disappeared.

From the above observations it was concluded that both aqueous and ethanolic extract of both the plants reversed the degenerative changes in liver structure however, the ethanolic extracts have more pronounced hepatoprotective effect similarly A. paniculata extracts administration reversed to large extent the hepatic lesions produced by thioacetamide in albino rat under present investigation than the extracts of E. viride. Hepatoprotective effects of ethanolic extract of A. paniculata leaf on diclofenac induced hepatotoxicity in rats have also been investigated previously by Darbar et al. (2009) and of crude ethanolic extract of A. paniculata on paracetamol induced mice by Devraj et al. (2010) and by Begum et al. (2011). Histopathological observations in above studies, the authors reported significant regenerative changes in the structure of liver in comparison with the toxicant induced animals after treated with the extracts of A. paniculata. Similarly, hepatoprotective and antioxidant effect of E. viride have also been studied by Cheedella et al. (2013) against paracetamol induced hepatotoxicity in albino Wistar rats and revealed that the ethanolic extract of E. viride has significant hepatoprotective activity against the paracetamol toxicity in rats and it may be due to their antioxidant property.

In phytochemical screening of the extracts of various plants by the earlier researches, they reported presence of constituents such as reducing sugars,
saponins, alkaloids, flavonoids, anthraquinine, tannins, terpenoids etc. According to all of these investigators the hepatoprotective effects of these plants largely depend upon these constituents. In the present investigation it is also considered that the *A. paniculata* and *E. viride* might contain such type of enzymatic and non-enzymatic antioxidant and the hepatoprotection might be due to these active ingredients of these plants that need further investigation.

**B. Kidney:**

Kidneys are the prime organs in the body. Kidney plays a role as eliminator, maintainer, regulator and also as a producer. As an eliminator, it helps to remove metabolic wastes products such as urea, ammonia and creatinine. Along with nitrogenous wastes, the kidney also excretes metabolites of xenobiotics. As a maintainer and regulator it maintains osmolarity of blood by regulating fluid balance and by maintaining acid base balance it regulates pH of the blood and blood pressure. Besides, kidneys also produce vitamin – D and some hormones that stimulates red cell production, regulates blood pressure and calcium metabolism (Kelly, 2004; Scanion and Sanders, 2003; National Kidney Federation, 2003). Kidney is more sensitive to toxic substances than any other organs in the body and is highly prone to toxic substances than any other organs in the body because large volume of blood flows through it and it filters large amount of toxins which can accumulate in the kidney tubules (Begum *et al*., 2011). It can result in systematic toxicity that decreases the ability of kidney to excrete body wastes, maintain body fluid, electrolyte balance and synthesis of the hormones (Oduol *et al.*, 2010).

As kidneys receive large volume of blood (about 20-25% of the cardiac output) consequently any toxic substance in the systemic circulation ultimately delivered to these organs in relatively high amount that can produce structural alternations and damage to these organs. Thioacetamide is one of such most potent nephrotoxic substance used in the present investigation to induce nephrosclerosis in experimental animals, albino rats. It exhibited several histopathological alterations in most of the components of nephron, the
structural and functional unit of kidney in rat intoxicated with it for about 4, 8 and 12 weeks. Significant and progressive degenerated changes were noticed with increase in period of intoxication and the degenerative changes were most significant in the kidney of rat intoxicated for 8 and 12 weeks of intoxication. The histopathological changes were characterized by enlargement of glomeruli, increased glomerular cellularity, tightly fitting Bowman’s capsule with no periglomerular space in few Bowman’s capsule while increased urinary space in some of Bowman’s capsule, elongation of tubules and disruption of tubular cells, accumulation of blood cells in lumen of ruptured tubules and vascular congestion, progressive pycnosis karyorrhexis and karyolysis with increase in period of intoxication, eosinophilic or hyaline cast formation in the lumen of necrosed tubules, sclerosed glomerulus, hyperplastic intimal necrosis or onion pell profilteration of arteriole, increased necrosis with fibrosis at some tubular region, elongation of medullary tubules, necrosis of tubular cells and complete destruction of cortical and medullary tubules with fibrosis. Glomerular conjugation, interstitial fibrosis and formation of scar tissue were observed as most significant histopathological alterations in kidney of rats intoxicated with TAA for 12 weeks.

An insight into existing literature revealed that number of earlier researchers have reported more or less similar histopathological lesions in kidney of experimental animal exposed to certain toxic substances. Aydin et al. (2003) invested effect of low medium and high doses of diclofenac sodium a non-steroidal anti-inflammatory drug on histopathology of kidney of rats. They found non-significant degenerative changes in the tubular epithelial cells and hyperemetic vessels in the interstitial areas both in cortex and medulla of kidney in rat those given low dose of drug. In the kidney of rat which were given medium dose of drug, besides focal tubular epithelial degeneration and destruction mild fibrosis was observed while cortical and medullary tubular epithelial degeneration, focal tubular necrosis, glomerular and tubular atrophy, increased fibrosis and infiltration of mononucler cells in the interstitium were noticed in kidney of rat intoxicated with higher dose of the same drug for the same period.
Observations and Discussion

of intoxication. Similarly, effects of lead exposure on kidney histopathology have been investigated by Missoun et al. (2010) in male Wistar rats. They reported general loss of cortical tissue, corticomedullary demarcation and vascular markings, small but intact pyramids, varying degrees of cellular interstitial nephritis, dilated tubules alternating with atrophic tubules, and varied degree of histopathological changes in the structure of glomeruli, including total destruction of some glomeruli, irregular distributed glomeruli with periglomerular fibrosis, nonspecific the abnormalities such as swelling and distortion of organelles in the cytoplasm of glomerular cells, adhesive glomerulitis with single adhesion to complete obliteration of the capsular space with intranuclear and cytoplasmic inclusion bodies. Ultrastructural changes like reduction in the number and size of the microvilli in the proximal convoluted tubular cells, shrinkage of nuclei, deformed mitochondria and folding of the cytoplasm, increased urinary space and formation of electron dense deposits in the basement membrane of the glomeruli have been reported by Tootian et al. (2012) in mice exposed to different concentration of phenol. Dose dependant histopathological alterations in the structure of kidney of albino rat intoxicated with gentamicin have been studied by Padmini and Kumar (2012). They observed epithelial cell degeneration and granular deposition in the lumen of proximal convoluted tubule with desquamation and lymphocytic infiltration, tubular epithelial necrosis, dilation of cortical tubules in albino rats treated with low dose treatment while at higher dose treatment cell shrinkage, cytoplasm eosinophilia, pycnotic nuclei, breaking of glomerular capillaries, glomerular congestion, disruption of glomerulus, vacuolar degeneration of tubular cells with hyaline cast formation. Rekha et al. (2013) have investigated histopathological effects of two different doses of Chlorpyrifos on kidney of adult Wistar albino rats of either sex treated for 1, 2, 4, 6 and 8 week. They found no histological alterations in kidney after 1 week treatment at low dose. At the same dose of pesticide from 2 to 8 week of intoxication they found shrinkage of glomerulus at initial stage of treatment, tubular dilation, hypertrophy of epithelial cells of tubules, degeneration of some tubules, infiltration of lymphocytes, increased
vascularity and interstitial edema, acute tubular necrosis and interstitial nephritis leading to acute renal failure and at longer treatment chronic renal failure. However at higher dose treatment histopathological alterations were noticed even after 1 week treatment and more pronounced alterations from 2 weeks to 8 week treatment. Effect of low, mild and higher dose of acrylamide histology, on kidney have been studied by Mahmood et al. (2015) in albino Wistar rats. They reported several histopathological alterations like degeneration of the glomerular tuft with lymphocytic infiltration, vacuolated renal tubules with rupture of the tubular epithelial cells, necrosis and congestion of the interstitial blood vessels. Dardouri et al. (2016) reported glomerular atrophy, dilation of the Bowman’s capsule, degeneration of tubular cells with pycnosis in kidney cortex of Wistar rats exposed to Cadmium.

Various plants have renoprotective activity. The extracts obtained from different parts of these plants have successfully been used traditionally to treat the patients suffering from kidney problems since long ago. Existing literature also revealed that number of earlier workers have investigated the renoprotective effects of various plant extracts against the kidney damage produced by certain toxic substances in the experimental animals.

Nephroprotective activity of aqueous seed extracts of *Carica papaya* have been tested by Olagunju et al. (2009) in *CCL₄* induced renal injury in Wistar rats; of crude ethanolic extract of root of *Croton zambesicus* in both male and female mice and rat by Okokon et al. (2011) against gentamicin induced kidney injury; of ethanolic extract of *Vitex negundo* by Kadir et al. (2013) on TAA induced nephrotoxicity in rats and of chloroform, ethyl acetate and ethanol extract of leaves of *Tephrosia purpurea* on gentamicin induced nephrotoxicity in rats by Jain et al. (2013). Renal protective activity of ethanolic leaf extract of *Adhatoda zeylanica* have been evaluated by Kumar et al. (2013) against gentamicin induced nephrotoxicity in Wistar rats; of mercury based Ayurvedic formulation (Sidh Makardhwaj) by Kumar et al. (2014) in rats; of ethanolic extract of leaves of *A. paniculata* by Padmalochana and Rajan (2015) against gentamicin
intoxicated adult male Wistar rats; of Olive and Juniper leaves extract individually and in combination by Al-Attar et al. (2015) against thioacetamide induced nephrotoxicity in male mice; of ethanolic extract of A. paniculata by Adejo et al. (2016) against CCl₄ induced nephrotoxicity in rat; of ethanolic extract of aerial part of Juniperus sabina L. by Abdel-Kader et al. (2017) against CCl₄ induced nephrotoxicity in male Wistar albino rats and of flax seed oil against thioacetamide induced renal toxicity in rats by Shaikh Omar (2018).

All above investigators finally reported that the extracts of the plants or plant materials they used significantly prevented histopathological alterations produced by particular toxicants towards normal as in control animal provide renoprotective effects. In the present investigation the aqueous and ethanolic extracts of A. paniculata and E. viride also exhibited renoprotective effects against thioacetamide induced nephrotoxicity in albino rat. This conclusion was drawn, as significant improvement in the intensity of histopathological alterations produced in the kidney of thioacetamide intoxicated rats noticed after treated these rats with aqueous and ethanolic extracts of both these plants. The kidney structure was observed as normal as in control animal.